DIAGNOSTIC HYBRIDS, INC.
350 West State Street
Athens, OHIO 45701

DIAGNOSTIC HYBRIDS
*Integrating Science and Humanity*™

SEP 2 1 2007

K063798

# 510(k) SUMMARY

## D3 HERPES SIMPLEX VIRUS IDENTIFICATION KIT

**Applicant**

DIAGNOSTIC HYBRIDS, INC.
350 West State Street
Athens, OHIO 45701

**Contact Information**

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**Date of preparation of 510(k) summary:**

December 18, 2006

**Device Name**

Trade name – $D^3$ HERPES SIMPLEX VIRUS IDENTIFICATION KIT
Common name – Fluorescent antibody test for herpes simplex virus
Classification name – ANTISERA, FLUORESCENT, HERPESVIRUS HOMINIS 1,2 (21 CFR 866.3305, product code GQL)

**Legally marketed devices to which equivalence is claimed:**

K902662 Bartels® Herpes Simplex Virus Fluorescent Monoclonal Antibody Test

K904167 Pathodx® Herpes Typing Kit

K880157 MicroTrak® HSV 1/HSV 2 Culture Identification and Typing Test

**Device Description**

The Diagnostic Hybrids D3 DFA Herpes Simplex Virus Identification Kit includes a DFA Reagent that contains a blend of four fluorescein-labeled murine monoclonal antibodies (MAbs), two directed against HSV type 1 (HSV-1) and two against HSV type 2 (HSV-2). The HSV-1 MAbs were developed using HSV-1(f) cell lysate as immunogen – one has been determined to be directed against HSV-1 glycoprotein C1, the antigen to the other is undetermined. The HSV-2 MAbs were developed using a HSV-2 recombinant glycoprotein G immunogen.

The kit includes the following components:

- HSV DFA Reagent – A blend of fluorescein labeled murine monoclonal antibodies directed against antigens produced in HSV-infected cell culture. The buffered, stabilized, aqueous solution contains Evan's Blue as a counter-stain and 0.1% sodium azide as preservative.
- HSV Antigen Control Slides - Individually packaged control slides containing wells with cell culture derived positive and negative control cells. Each HSV positive well is identified. The negative wells contain uninfected cells. Each slide is intended to be stained only one time.

• PBS Concentrate - A 40X concentrate consisting of 4% sodium azide in phosphate buffered saline (after dilution to 1X in water, the concentration of sodium azide in the solution is 0.1%).

• Mounting Fluid - an aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.

The cells to be tested, on a slide prepared from a tube culture or on a monolayer of cells cultured in a multi-well plate or a coverslip in a shell vial, are fixed in acetone. The HSV DFA Reagent is added to the cells to detect the presence of HSV specific viral antigens. After incubating for 15 to 30 minutes at 35° to 37°C, the stained cells are washed with the supplied Phosphate Buffered Saline (PBS). To prepare the slide for examination, a drop of the supplied Mounting Medium is added to the stained cells and a coverslip is placed on the slide. To prepare the centrifuge enhanced cell cultures for examination, a drop of Mounting Fluid is placed on a clean microscope slide. The coverslip is removed from the shell vial and placed on to the Mounting Fluid.

For multi-well plates, monolayers are fixed with an 80% aqueous acetone solution. The HSV DFA Reagent is added to the cells to detect the presence of any HSV specific viral antigens. After incubating for 15 to 30 minutes at 35° to 37°C, the stained cells are washed with the supplied Phosphate Buffered Saline (PBS). Mounting Fluid is added to each well to cover the monolayers.

The slides or wells are examined using a fluorescence microscope equipped with the correct filter combination for FITC at a magnification of 100-400X. Virus infected cells will be stained with bright apple-green fluorescence while uninfected cells will contain no apple-green fluorescence but will fluoresce red by the Evan's Blue counter-stain[1] which is included in the HSV DFA Reagent.

If no fluorescent cells are found, report result as, "No herpes simplex virus detected". If fluorescent cells are found, report result as, "Herpes simplex virus isolated by cell culture."

Included in the kit are HSV Antigen Control Slides. A Control Slide is intended to function as an indicator that the kit reagents are working properly in the test. [The slides are prepared with wells of HSV infected cells and uninfected cells.] Positive and negative controls must demonstrate appropriate staining characteristics for specimen results to be valid. Controls may also aid in the interpretation of test results.

It is recommended that cell culture positive (infected with known HSV isolate) and negative (uninfected cells) controls be run with each assay to provide a means to ensure adequate performance of the cell culture system used. If control cultures fail to perform correctly, results are considered invalid.

**Intended Use**

The Diagnostic Hybrids D3 DFA Herpes Simplex Virus Identification Kit is intended for use in the qualitative detection of human herpes simplex virus (HSV) in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs). Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

Performance using direct specimen testing has not been evaluated.

**Explanation**

HSV infections in humans can cause lesions at a variety of sites, e.g., oral-facial, genital, eye, and cutaneous sites.

When an appropriately sensitive cell line is infected with HSV, a characteristic deterioration of cells, termed cytopathic effect (CPE), can be observed. Tube culture, a classic format for virus amplification, can take several days before CPE is evident. In the case of those specimens with low titers of virus, 7 days of culture may be required by the standard tube culture method before CPE can be observed[2,3,4,5,6].



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The rate of isolation may be enhanced and the time required for HSV identification may be decreased by centrifugation of specimens in shell vials or multi-well plates containing appropriately sensitive cell lines (centrifuge enhanced technique) [7,8,9].

Even so, CPE may be difficult to interpret due to, for instance, deterioration of cells, which can result from toxic components present in the clinical specimen making microscopic examination of the infected cells problematic. Because of this, immunofluorescence confirmation of cell culture is regarded as the standard for confirmation of a HSV positive result.

**Technological Characteristics**

Fundamental technology and intended use of the device are the same as those of the predicate devices, which are based on a standard immunofluorescence assay technique using cells inoculated with patient specimens. They employ directly labeled fluorescein monoclonal antibodies specific for HSV antigens enabling visualization of the infected cells. A summary is provided in the table below:

| HSV Systems | DFA | Direct Specimens | Culture Confirmation | FITC Label | Monoclonal Antibody | Distinguishes HSV-1 and HSV-2 |
|---|---|---|---|---|---|---|
| Diagnostic Hybrids | Yes | No | Yes | Yes | Yes | No |
| Bartels® (Trinity) | Yes | No | Yes | Yes | Yes | No |
| PathoDx (Remel) | Yes | Yes | Yes | Yes | Yes | Yes |
| MicroTrak® (Trinity) | Yes | No | Yes | Yes | Yes | Yes |

**Non-clinical Performance**

Staining patterns of the conjugated monoclonal antibodies on HSV infected cells were similar to those of the predicate devices.

Analytical specificity was evaluated by staining cultures infected with a number of ATCC reference HSV-1 and HSV-2 strains and found to react with all of them.

The HSV DFA Reagent was tested for cross-reactivity against a wide variety of other microorganisms and cells. No cross-reactivity was observed for 59 virus strains (cultured and processed for staining) or for 17 host culture cell types. Twenty-seven (27) bacterial cultures, one yeast and one protozoan culture were stained and examined for cross-reactivity, including Staphylococcus aureus, a protein-A-producing bacterium. Staining of S. aureus appeared as small points of fluorescence while all other cultures were negative. [Protein A will specifically bind to the Fc portions of conjugated antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, i.e., S. aureus-bound fluorescence appears as small (~1 micron diameter), bright dots.] **[Note: Results from cell cultures with bacterial contamination must, therefore, be interpreted with caution.]**

Stringent conditions for cross-reactivity testing were achieved by using a high concentration of the HSV DFA Reagent and high titers of microorganisms. The DFA Reagent was prepared at 1.5X the concentration that is provided in the kit.

Depending on the particular virus, 500 to 715 $TCID_{50}$ viruses were inoculated into shell vial or multi-well plate cultures and incubated for 24 to 48 hours to yield a 1+ to 3+ infection, processed and stained with the 1.5X DFAs according to the procedure detailed in the product insert. Stained cells were examined at 200x magnification. Cell cultures were stained as confluent monolayers.

Bacteria and yeast were cultured, processed as suspensions, then spotted on microscope slides (at CFUs ranging from 6.4x104 to 2.93x107/well in a 10 µL dot, depending on the bacterium), then stained with the 1.5X DFAs according to the procedure in the product insert. Stained slides were examined at 400X magnification.



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Some microorganisms were procured from an external source as prepared microscope slides, intended to be used as positive controls for assays.

**Virus Strains Tested for Cross Reactivity with D³ HSV DFA Reagent**

| Organism | Strain or Type | Inoculum ($TCID_{50}$) |
|---|---|---|
| Adenovirus | Type 1 | 715 |
| Adenovirus | Type 3 | 715 |
| Adenovirus | Type 5 | 715 |
| Adenovirus | Type 6 | 715 |
| Adenovirus | Type 7 | 715 |
| Adenovirus | Type 8 | 715 |
| Adenovirus | Type 10 | 715 |
| Adenovirus | Type 13 | 715 |
| Adenovirus | Type 14 | 715 |
| Adenovirus | Type 18 | 715 |
| Adenovirus | Type 31 | 715 |
| Adenovirus | Type 40 | 715 |
| Adenovirus | Type 41 | 715 |
| Influenza A | Aichi | 715 |
| Influenza A | Mal | 715 |
| Influenza A | Hong Kong | 715 |
| Influenza A | Denver | 715 |
| Influenza A | Port Chalmers | 715 |
| Influenza A | Victoria | 715 |
| Influenza A | New Jersey | 715 |
| Influenza A | PR | 715 |
| Influenza A | WS | 715 |
| | | |
| Echovirus | Types 4, 6, 9, 11, 30, 34 | Commercially available slides stained.[1] |
| Coxsackievirus | Types B1, B2, B3, B4, B5, B6 | Commercially available slides stained.[1] |

| Organism | Strain or Type | Inoculum ($TCID_{50}$) |
|---|---|---|
| Influenza B | Hong Kong | 715 |
| Influenza B | Maryland | 715 |
| Influenza B | Mass | 715 |
| Influenza B | Taiwan | 715 |
| Influenza B | GL | 715 |
| Influenza B | JH-001 isolate | 715 |
| Influenza B | Russia | 715 |
| RSV | Long | 715 |
| RSV | Wash | 715 |
| RSV | 9320 | 715 |
| Parainfluenza 1 | C-35 | 715 |
| Parainfluenza 2 | Greer | 715 |
| Parainfluenza 3 | C 243 | 715 |
| Parainfluenza 4a | M-25 | 715 |
| Parainfluenza 4b | CH19503 | 715 |
| CMV | Towne | 700 |
| CMV | Davis | 700 |
| CMV | AD169 | 700 |
| VZV | Webster | 500 |
| VZV | Allen | 500 |
| Epstein-Barr | Commercially available slides stained.[a] | |
| Rubeola | | |
| Mumps | | |
| HPV | Types 6, 11 | Commercially available slides stained.[1] |

**Cell Lines Tested for Cross Reactivity with D³ HSV DFA Reagent**

| | | |
|---|---|---|
| A-549 | MRHF | RhMK II |
| BGMK | Mv1Lu | pRK |
| HEp-2 | NCI-H292 | RD |
| LLC-MK2 | pCMK | R-Mix |
| MDCK | pRhMK | Vero |
| MRC-5 | | WI-38 |

**Microorganisms Tested for Cross Reactivity with D³ HSV DFA Reagent**

| BACTERIA | CFU TESTED | BACTERIA | CFU TESTED |
|---|---|---|---|
| Acinetobacter calcoaceticus | $9.7 \times 10^5$ | Salmonella typhimurium | $1.7 \times 10^6$ |
| Bordetella bronchiseptica | $1.7 \times 10^5$ | Staphylococcus aureus | $1.0 \times 10^7$ |
| Bordetella pertussis | $4.6 \times 10^6$ | Streptococcus agalactiae | $9.6 \times 10^6$ |
| Corynebacterium diphtheriae | $2.5 \times 10^6$ | Streptococcus pneumoniae | $8.0 \times 10^5$ |
| Escherichia coli | $2.6 \times 10^5$ | Streptococcus pyogenes | $2.9 \times 10^7$ |
| Gardnerella vaginalis | $5.0 \times 10^5$ | Acholeplasma laidlawi | $\sim 6 \times 10^7$ |
| Haemophilis influenzae type A | $9.3 \times 10^5$ | Mycoplasma hominis | $\sim 6 \times 10^4$ |
| Klebsiella pneumoniae | $6.4 \times 10^6$ | Mycoplasma orale | $\sim 6 \times 10^4$ |
| Legionella pneumophila | $6.5 \times 10^4$ | Mycoplasma pneumoniae | $\sim 6 \times 10^4$ |
| Moraxella cartarrhalis | $6.4 \times 10^4$ | Mycoplasma salivarium | $\sim 6 \times 10^7$ |
| Neisseria gonorrhoeae | $1.3 \times 10^6$ | Ureaplasma uralyticum | $\sim 6 \times 10^4$ |
| Proteus mirabilis | $2.1 \times 10^6$ | Chlamydophila pneumoniae | Commercially available slides stained.[1] |
| Pseudomonas aeruginosa | $1.0 \times 10^7$ | Chlamydia trachomatis | Commercially available slides stained.[1] |
| Salmonella enteriditis | $2.5 \times 10^6$ | | |
| | | | |

[a] Test material is from commercially available prepared slides. Each positive well contained 10% to 50% reactive cells.



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| | | | |
|---|---|---|---|
| YEAST | | PROTOZOAN | |
| Candida glabrata | $8.7 \times 10^6$ | Trichomonas vaginalis | [Commercially available slides stained.][1] |

**Clinical Performance**

Clinical studies have been conducted at four different laboratories where they compared the D3 DFA HSV Kit performance to that of comparison tests using five hundred and thirty (530) specimens. A combination of fresh (250) and frozen (280) specimens were tested. Three specimens from site 4 were not evaluated due to bacterial contamination of the monolayers, leaving 527 for analysis.

Two study sites used tube cultures, one used shell vial culture, and one used multi-well plates. Specimens were processed and cultured according to each laboratory's established procedures and testing performed according to the respective tests' instructions for use. The resulting stained cells were microscopically evaluated and results reported as positive or negative for identification of HSV.

Positive and Negative Percent Agreement between the Subject and Comparison test results were calculated and reported at 95% confidence interval.

Table of Combined Specimen Results from Four Study Sites

| | | Comparison Device + | Comparison Device - |
|---|---|---|---|
| Subject Device Diagnostic Hybrids | + | 200 | 1 |
| | - | 1 | 325 |
| Positive Percent Agreement [b] (PPA) | | 99.5% | |
| 95% CI [c] – PPA | | 97.3% - 100% | |
| Negative Percent Agreement [d] (NPA) | | 99.7% | |
| 95% CI – NPA | | 98.3% - 100% | |

[1] Specter, S., Hodinka, R. L., and Young, S.A. 2000, Clinical Virology Manual, Washington D.C., ASM Press, 420-424.

[2] Bryson, Y.J., M. Dillon, M. Lovett, G. Acura, S. Taylor, J.D. Cherry, L. Johnson, E. Weismeier, W. Growdeon, T. Creagh-Kirk and R. Keeney. 1983. "Treatment of first episodes of genital *Herpes simplex* virus infection with oral acyclovir: A randomized double-blind controlled trial in normal subjects". *New Eng.* J. *Med.,* 308: 916-921.

[3] Dulbecco, R. and H.S. Ginsberg. 1973. "Herpesviruses", Chapter 55 in *Microbiology,* Third Edition, D.D. Davis, et al. (eds.), Harper and Row, Hagerstown.

[4] Gleaves, C.A., D.J. Wilson, A.D. Wold and T.F. Smith. 1985. "Detection and Serotyping of *Herpes simplex* Virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16h post-inoculation". J. *Clin. Micro.,* 21: 29-32.

[5] Reeves, E.C., L. Corey, H.G. Adams. L.A. Vontver and K.K. Holmes. 1981. "Risk of recurrence after first episodes of genital herpes: relation to *HSV* type and antibody response". *New Eng.* J. *Med.,* 305: 315-319.

[6] Ashley, R.L., L. Corey, and W. E. Rawls. 1989. *"Herpes simplex* Viruses", Chapter 11 in *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections.* Sixth Edition, N.J. Schmidt and R.W. Emmons, (eds), American Public Health Association, Inc., Washington, D.C.

[7] Gleaves, C.A., D.J. Wilson, A.D. Wold and T.F. Smith. 1985. "Detection and Serotyping of *Herpes simplex* Virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16h post-inoculation". J. *Clin. Micro.,* **21**: 29-32.

[8] Moore, D.F. 1984. "Comparison of human fibroblast cells and primary rabbit kidney cells for isolation of *Herpes simplex* virus". J. *Clin. Micro.* **19:** 548-549.

[9] Ashley, R.L., L. Corey, and W. E. Rawls. 1989. *"Herpes simplex* Viruses", Chapter 11 in *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections.* Sixth Edition, N.J. Schmidt and R.W. Emmons, (eds), American Public Health Association, Inc., Washington, D.C.

[b] "Positive Percent Agreement", or "PPA", values were calculated according to {[Total Number of Positive Results in Agreement by both Subject and Predicate Tests) divided by [(Total Number of Positive Results in Agreement by both Subject and Predicate Tests) plus (Number of Results Positive by Predicate but Negative by Subject)]} multiplied by 100%.

[c] "95% CI" refers to 95% Confidence Intervals, which were calculated according to Exact method (Clopper, C. and S. Pearson, Biometrika 26:404-413, 1934).

[d] "Negative Percent Agreement", or "NPA", values were calculated according to {[Total Number of Negative Results in Agreement by both Subject and Predicate Tests) divided by [(Total Number of Negative Results in Agreement by both Subject and Predicate Tests) plus (Number of Results Negative by Predicate but Positive by Subject)]} multiplied by 100%.



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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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Rockville MD 20850

SEP 2 1 2007

Ms. Gail R. Goodrum
Vice President, Regulatory and Quality Affairs
Diagnostic Hybrids, Inc.
350 West State Street
Athens, OH 45701

Re: k063798
Trade/Device Name: Diagnostic Hybrids $D^3$ DFA Herpes Simplex Virus Identification Kit
Regulation Number: 21 CFR § 866.3350
Regulation Name: Herpes simplex virus serological reagents
Regulatory Class: II
Product Code: GQN
Dated: August 10, 2007
Received: August 13, 2007

Dear Ms. Goodrum:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at 240-276-0450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at 240-276-3474. For questions regarding the reporting of device adverse events (Medical Device Reporting (MDR)), please contact the Division of Surveillance Systems at 240-276-3464. You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

# Indications for Use

510(k) Number (if known): K063798

Device Name: Diagnostic Hybrids $D^3$ DFA Herpes Simplex Virus Identification Kit

Indications for Use: The Diagnostic Hybrids $D^3$ DFA Herpes Simplex Virus Identification Kit is intended for use in the qualitative detection of human herpes simplex virus (HSV) in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs). Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.
Performance using direct specimen testing has not been evaluated.

Prescription Use X (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE OF NEEDED)

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Concurrence of CDRH, Office of Device Evaluation (ODE)

Division Sign-Off

**Office of In Vitro Diagnostic Device Evaluation and Safety**

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